This is a digital copy of a book that was preserved for generations on library shelves before it was carefully scanned by Google as part of a project to make the world's books discoverable online.

It has survived long enough for the copyright to expire and the book to enter the public domain. A public domain book is one that was never subject to copyright or whose legal copyright term has expired. Whether a book is in the public domain may vary country to country. Public domain books are our gateways to the past, representing a wealth of history, culture and knowledge that's often difficult to discover.

Marks, notations and other marginalia present in the original volume will appear in this file - a reminder of this book's long journey from the publisher to a library and finally to you.

Usage guidelines

Google is proud to partner with libraries to digitize public domain materials and make them widely accessible. Public domain books belong to the public and we are merely their custodians. Nevertheless, this work is expensive, so in order to keep providing this resource, we have taken steps to prevent abuse by commercial parties, including placing technical restrictions on automated querying.

We also ask that you:

+ *Make non-commercial use of the files* We designed Google Book Search for use by individuals, and we request that you use these files for personal, non-commercial purposes.

+ *Refrain from automated querying* Do not send automated queries of any sort to Google's system: If you are conducting research on machine translation, optical character recognition or other areas where access to a large amount of text is helpful, please contact us. We encourage the use of public domain materials for these purposes and may be able to help.

+ *Maintain attribution* The Google "watermark" you see on each file is essential for informing people about this project and helping them find additional materials through Google Book Search. Please do not remove it.

+ *Keep it legal* Whatever your use, remember that you are responsible for ensuring that what you are doing is legal. Do not assume that just because we believe a book is in the public domain for users in the United States, that the work is also in the public domain for users in other countries. Whether a book is still in copyright varies from country to country, and we can't offer guidance on whether any specific use of any specific book is allowed. Please do not assume that a book's appearance in Google Book Search means it can be used in any manner anywhere in the world. Copyright infringement liability can be quite severe.

About Google Book Search

Google's mission is to organize the world's information and to make it universally accessible and useful. Google Book Search helps readers discover the world's books while helping authors and publishers reach new audiences. You can search through the full text of this book on the web at `http://books.google.com/`

JAN 20 1921
QP
331
.G82

The University of Chicago

A QUANTITATIVE STUDY OF THE EFFECTS PRODUCED ON MOTOR NERVE OF FROG BY SALTS OF SODIUM, POTASSIUM, RUBIDIUM, AND CALCIUM

A DISSERTATION
SUBMITTED TO THE FACULTY
OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY

BY
ESTHER Maud GREISHEIMER

Private Edition, Distributed By
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
American Journal of Physiology, Vol. 49, No. 4
September 1, 1919

JAN 20 1921
QP
331
.G82

The University of Chicago

A QUANTITATIVE STUDY OF THE EFFECTS PRODUCED ON MOTOR NERVE OF FROG BY SALTS OF SODIUM, POTASSIUM, RUBIDIUM, AND CALCIUM

A DISSERTATION

SUBMITTED TO THE FACULTY
OF THE OGDEN GRADUATE SCHOOL OF SCIENCE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY

BY

ESTHER GREISHEIMER

Private Edition, Distributed By
THE UNIVERSITY OF CHICAGO LIBRARIES
CHICAGO, ILLINOIS

Reprinted from
American Journal of Physiology, Vol. 49, No. 4
September 1, 1919

Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY, Vol. 49, No. 4
September, 1919

[illegible] 5-9-29 [illegible]

A QUANTITATIVE STUDY OF THE EFFECTS PRODUCED BY SALTS OF SODIUM, POTASSIUM, RUBIDIUM AND CALCIUM ON MOTOR NERVE OF FROG

ESTHER GREISHEIMER

From the Hull Laboratories of Physiological Chemistry and Pharmacology, University of Chicago

Received for publication June 3, 1919

INTRODUCTION

Mathews (1) in the course of his investigations on the nature of chemical and electrical stimulation found the chloride of rubidium to be the most powerful stimulating agent for motor nerve of frog. Owing to conflicting reports in the literature on the subject, further investigation seemed desirable.

A brief review of the physiological effects of the rubidium salts and the related sodium and potassium salts will be given. Bunsen discovered rubidium in 1861 and knew from the chemical behavior of its compounds that it belonged in the group of alkali metals. In most of its peculiarities rubidium exhibits the greatest similarity to potassium, which it follows in the periodic system. Grandeau (2), however, as early as 1864 concluded that rubidium was more nearly related to sodium than to potassium. He injected 1 gram of the salt dissolved in 15 cc. of water into dogs. If the dog died the salt was considered toxic, but if the animal recovered sufficiently to run about the salt was considered ineffective. Grandeau, by these methods, found potassium alone to be toxic, and sodium and rubidium chlorides to be ineffective, in equal dosage.

Ringer (3) made a comparative study of these chlorides, using the ventricle of the frog's heart, in which he maintained an artificial circulation. On using 1 cc. of a one and two hundred five thousandths (1.205) per cent solution of rubidium chloride in 100 cc. of normal saline solution, he observed the ventricular contractility to grow less and less until it had disappeared. On addition of small amounts of either strontium or calcium chloride (3.5 to 5 cc. of 1 per cent sol. to 100 cc. of the circulating fluid) fair contractions speedily returned. A calcium salt in saline broadened the beat, rounded its top and greatly retarded dilatation. Rubidium, like potassium, obviated these effects, and restored to the beat its normal character.

Blake (4) criticised the work of Ringer because it had been done on the isolated organs of a dead animal. He believed that the physiological action of a salt should be determined by introducing it directly into the veins or arteries of a living animal, so that it could, with the least disturbance possible, be brought into direct contact with tissues whose reactions he wished to investigate. He injected the chlorides of rubidium, potassium and sodium into the veins or arteries of frogs in order to test the effects on the heart. His results differed from those of Ringer in that he found sodium and rubidium to behave similarly and potassium to be exceptional in its behavior, but he failed to give the data obtained.

Harnack and Dietrick (5) injected rubidium chloride subcutaneously into frogs, and compared the results with those following the injection of potassium chloride. These salts did not essentially affect the heart muscle when given in doses of about thirty milligrams. From the results obtained they concluded that the differences between potassium and rubidium salts were quantitative, potassium being the stronger of the two. A few investigations were carried out in which isolated muscle was placed in rubidium or potassium chloride, and a rapid decrease in height of contraction following stimulation followed in either salt. These investigators gave no data from experiments on motor nerve of frog, but simply stated that rubidium salts appeared to affect motor nerve to a slight degree only.

Brunton and Cash (6) observed that "motor nerve was not paralyzed by rubidium except in very large doses." The height of contraction of muscle was increased by rubidium and potassium alike. The lethal activity on frogs of the chlorides used placed the metals in the following series, potassium being the strongest: potassium > rubidium > barium > caesium > lithium > strontium > sodium > calcium.

Richet (7) studied the effects of both subcutaneous and intravenous injections of rubidium and potassium chlorides on fish, frogs, guinea pigs, pigeons and rabbits. The results were similar on the various animals, rubidium chloride being slightly less toxic than potassium chloride.

Botkin (8) injected these chlorides into dogs and found that rubidium chloride exerted effects similar to smaller doses of potassium chloride.

Grützner (9) has, as had Ranke (10) and Biedermann (11) before him, found the chloride of potassium, even in dilute solution, intensely toxic. In stimulating action, caesium was found most active, rubidium next, and potassium had no noticeable stimulating action at all. But in toxicity potassium stood highest, rubidium next and caesium lowest. Calcium also was found to be toxic to the nerve.

Many investigations on the antagonisms of these salts have been carried out. Loeb (12) found pure sodium chloride a strong poison for many (if not all) marine animals. He considered the poisonous effects due to the sodium ion. Equimolecular solutions of calcium and potassium chlorides were also found poisonous, but a combination of the three ions was not poisonous. Loeb and Cattell (13) showed that eggs of Fundulus poisoned with potassium chloride were unable to recover when placed in distilled water or in a saccharose solution, while they recovered when placed in an $\frac{M}{8}$ salt solution, or in acidified water. The relative efficiency of the salts for inducing the recovery of the heart beat increases, first with the concentration of the salt in the solution, then with the valency of the anion of the salt, the valency effect apparently following Hardy's rule. Loeb and Ewald (14) studied the inhibitory effect of calcium on motor nerve of frog. A portion of nerve immersed in an $\frac{M}{4}$ sodium chloride solution results in twitching of the attached muscle. They found that 2 cc. of an $\frac{M}{8}$ calcium chloride solution per 100 cc. of a $\frac{M}{4}$ sodium chloride are required to suppress these twitchings. On adding a quantity of calcium not quite high enough to inhibit entirely the effect of the stimulating salt, the latent period of stimulation was considerably increased. This fact harmonized with Loeb's assumption that the inhibitory effect of calcium is due to a prevention or a retardation of the diffusion of the stimulating salt into the nerve.

Burridge (15) perfused isotonic sodium chloride through the frog's heart. Stoppage in the dilated condition and loss of electrical irritability followed. Calcium opposed these effects.

Zoethout (16) found that gastrocnemii of frogs were thrown into contraction on immersion in $\frac{M}{8}$ potassium chloride. He showed that

METHODS

The sciatic nerve and the gastrocnemius muscle of the leopard frog were used. The sciatic was not cut, the whole sacral plexus with an attached bit of spinal column being left intact. It was believed that more constant results could be obtained if nerves were kept as nearly uninjured as possible. The frogs were brought from the tank and the preparation rapidly made, scarcely five minutes elapsing between the pithing of the frog and the mounting of the preparations in the moist chambers. The fact that frogs are in varying conditions of health is a great uncontrollable source of error. Sex appears to make no difference in the results, but the writer believes that humidity will in the future be shown to be an influential factor in nerve work.

A 4 cm. length of nerve nearest the muscle was immersed in a paraffin cup containing 5 cc. of the solution whose effects were to be studied. The nerve as it emerged from the solution was led across electrodes. At first platinum electrodes were used, but it was found that copper electrodes gave results identical with those obtained from the platinum electrodes, hence the use of copper ones was continued. This is contrary to general opinion, but control nerves remaining in Ringer's solution showed practically no decrease in irritability, even after twenty hours, so injury from copper electrodes did not occur to any extent. The remaining portion of the nerve with the attached bit of spinal column was placed in a second paraffin cup containing Ringer's solution. The entire nerve varied from 7 to 10 cm. in length. The cover of the moist chamber was not removed during the experiment, which was often continued for sixteen hours. Every known precaution was taken for keeping the conditions as favorable as possible for the nerve, and as a result the controls in Ringer's solution, run with the daily series of observations, showed very little change, if any, in irritability, even when the experiments were continued over a space as great as eighteen or twenty hours.

Two Edison primary batteries attached in parallel, and renewed from time to time, furnished the current for the primary coil. The coil used at the University of Chicago was of the small type for laboratory use, put out by C. H. Stoelting Company, Chicago. The greatest distance between coils on this type was 21.4 cm. The coil used at the University of Minnesota was put out by W. Oehmke, Berlin. The coils could be separated as much as 75 cm. with this type. Various types of keys were tried out, and the one most suitable was an ordinary wall-switch, conveniently mounted, which apparently gave a constant break shock. The greatest distance between the coils just effecting stimulation was determined at regular intervals. The increase in the strength of shock, as thus determined, was assumed to be a measure of the toxicity of the salt. The writer recognizes the criticisms of such an assumption, but knows of no improved method at present.

The salts were repeatedly recrystallized and the purity carefully tested. Redistilled water from a block tin condenser was used in making up the solutions. The data for purification are given in detail. The calcium chloride, C. P., was not recrystallized. About 45 grams were dissolved and made up to 250 cc. Then 10 cc. of this solution were diluted to 250 cc., and 50 cc. portions were evaporated and dried to constant weight in Jena dishes. Two samples weighed 0.3114 and 0.3116 gram respectively. This material was then redissolved, heated, and ammonium hydroxide and ammonium oxalate added. The material was kept on the steam bath over night. After filtering and washing, the precipitate was ignited to CaO in platinum crucibles. On drying to constant weight, the CaO weighed 0.1569 and 0.1568 respectively. Then 111 : 56.1 :: X : 0.1568 X = 0.3102 gram $CaCl_2$. The actual weight of $CaCl_2$ was 0.3114, the calculated weight 0.3102 gram, therefore, the impurity was 0.3 per cent.

The potassium chloride had been recrystallized by Miss M. Koch. About 38 grams were dissolved and made up to 250 cc. Of this, 10 cc. were diluted to 250 cc., and then 25 cc. portions taken for analysis. These were evaporated and dried to constant weight in Jena dishes, and two samples weighing respectively 0.1351 and 0.1352 gram were used. This material was then dissolved, potassium chromate added, and titrated with standard $AgNO_3$. The amounts of $AgNO_3$ required were 11.27 cc. and 11.28 cc. respectively. The factor of the $AgNO_3$ was 1.607. Then $11.27 \times 1.607 \times 0.00746 = 0.1351$ gram; $11.28 \times 1.607 \times 0.00746 = 0.1352$ gram. As these were identical with the actual weight, the salt was 100 per cent pure.

The sodium chloride was recrystallized four times. Five cubic centimeters of the stock solution were diluted to 250 cc., and 25 cc. samples were taken. The two samples were evaporated in Jena dishes and dried to constant weight. They weighed 0.0891 gram. This material was dissolved and titrated with $AgNO_3$, using potassium chromate as an indicator. The amount of $AgNO_3$ required was 9.58 cc. Then $9.58 \times 1.607 \times 0.00585 = 0.0900$ gram. The actual weight was 0.0891 gram, hence there was 1.0 per cent impurity.

The sodium bromide was recrystallized four times. About 28 grams were dissolved and made up to 250 cc. Of this stock solution 10 cc. were diluted to 250 cc., and samples of 25 cc. were taken. The samples weighed 0.1093 gram, on evaporating and drying to constant weight. The material was dissolved and titrated with $AgNO_3$, 6.79 cc. being required for the titrations. $6.79 \times 1.607 \times 0.0103 = 0.1123$ gram. The actual weight was 0.1090, hence the impurity was 2 per cent.

The sodium sulphate was recrystallized four times. About 75 grams were dissolved and made up to 500 cc. Of this, 10 cc. were diluted to 250 cc., and 25 cc. were evaporated and dried to constant weight. The samples weighed 0.1417 gram. This was dissolved, heated and precipitated by adding $BaCl_2$. The precipitate was filtered, washed and ignited to constant weight. The actual weight of $BaSO_4$ was 0.2335 gram. The calculated weight $-(142.16 : 233.46 :: 0.1417 : X)$ was 0.2327. The impurity was therefore equal to 0.3 per cent.

The rubidium chloride was made from rubidium carbonate. After several recrystallizations, about 32 grams were dissolved and made up to 250 cc. Of this 1 cc. portions were evaporated and dried to constant weight. The samples weighed 0.1261 gram. This was dissolved and titrated with $AgNO_3$; 10.59 cc. were required for the titration. The factor for this $AgNO_3$ was 0.985. $10.59 \times 0.985 \times 0.012095 = 0.1261$ gram hence the salt was 100 per cent pure.

The rubidium bromide was recrystallized three times. About 45.5 grams were dissolved and made up to 250 cc. Of this 1 cc. portions evaporated and dried to constant weight. The samples weighed 0.1787 gram. This material was dissolved and titrated with $AgNO_3$, using 11.27 cc. in the titration. $11.27 \times 0.985 \times 0.016546 = 0.1836$ gram. Hence the impurity of the rubidium bromide was 2.7 per cent.

The rubidium sulphate was recrystallized three times. Five cubic centimeter portions of the stock solution were taken, evaporated and dried to constant weight. The samples weighed 0.1807 gram. This material was dissolved and precipitated with $BaCl_2$. The precipitate, after standing on the steam bath for twenty-four hours, was filtered off, washed and ignited to constant weight. The $BaSO_4$ weighed 0.1568 gram. The theoretical weight was $(267.06 : 233.46 :: 0.1807 : X)$ 0.1579 gram. Hence the impurity of the salt was 0.7 per cent. This is given in detail because the writer feels that physiologists generally do not realize the importance of having pure salts to start with.

The salts most extensively studied were the chlorides of sodium, calcium, rubidium and potassium. Fewer observations were made on sodium and rubidium sulphates and sodium and rubidium bromides. The sulphates of sodium and rubidium and the chloride of calcium were used in twelfth-molar concentration, but all others were used in an eighth-molar concentration, unless otherwise stated. Assuming that salts composed of a monovalent cation and a monovalent anion dissociate into two ions, and those composed of either a divalent cation or a divalent anion dissociate into three ions (as $CaCl_2$ or Na_2SO_4) an eighth-molar solution of the former is approximately isotonic with a twelfth-molar solution of the latter. The solutions must be isotonic rather than equimolecular, for work on nerve.

In the early period of the work records were kept of the muscular twitchings, but these were discontinued after a time, since they gave no additional information. The results of a large number of experiments were averaged. In spite of the current opinion that there is nothing constant about nerve work, the writer believes that a fair degree of constancy may be obtained if reasonable precautions are taken.

RESULTS

1. Isotonic salt solutions

Sodium salts. With $\frac{M}{8}$ sodium chloride the latent period was two to three hours, and the period of contractions lasted about three hours. The contractions occurred as single twitches, and relaxation followed each contraction, with short periods of inactivity between the successive twitches. The irritability dropped considerably at first, then increased again, before the final fall. Fifty-two experiments were done for the three-hour period. As these were done at the University of Chicago, they were tested by the small induction coil, which was arranged to read at a maximum of only 21.4 cm. between the coils. Of course, no decrease in irritability was shown by this coil, so a straight line may represent the results, as shown by curve A, figure 1.

Quite a different picture is obtained when a larger coil is used. The coil used for the experiments carried out at the University of Minnesota was arranged to allow a maximum distance of 75 cm. between the coils. Twenty-five experiments were carried out with this coil, and table 1 shows the results for a period of twenty-two hours:

TABLE 1

A	0	½	1	2	3	5	7	8	9	13	22
B	63.3	31.8	24.7	27.6	39.4	43.9	61.7	40.5	39.1	31.7	12.3
C	68.0	62.5	26.5	33.5	68.0	73.0	71.0	72.0	75.0	39.2	31.0
D	57.5	22.0	21.0	20.5	15.0	13.5	45.7	8.3	7.5	22.7	5.0

As it is impossible to give all the readings for all the nerves, only the average, the maximum and the minimum readings are given in the table.

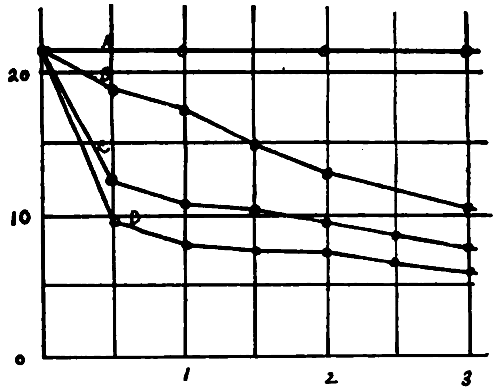

Fig. 1. A = $\frac{M}{8}$ NaCl; B = $\frac{M}{12}$ $CaCl_2$, table 4; C = $\frac{M}{8}$ KCl, table 6; D = $\frac{M}{8}$ RbCl, table 8. Abscissae—time in hours. Ordinates—distance in cms. between coils.

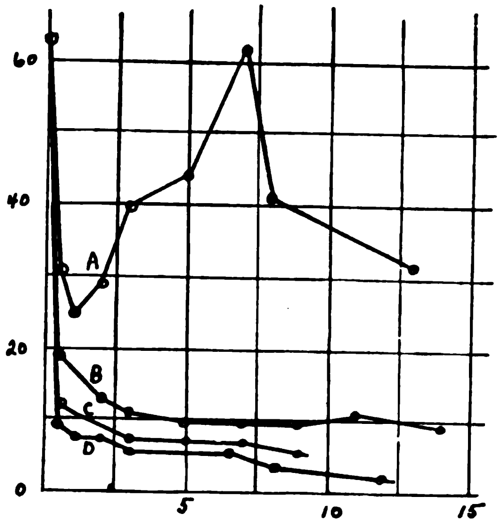

Fig. 2. A = $\frac{M}{8}$ NaCl, table 1; B = $\frac{M}{12}$ $CaCl_2$, table 5; C = $\frac{M}{8}$ KCl, table 7; D = $\frac{M}{8}$ RbCl, table 9.

A indicates the time in hours; B, the average readings of the twenty-five experiments; C, the maximum reading of the nerve showing the greatest irritability; D, the minimum reading of the nerve showing the least irritability. The extremes are variable, as shown by the table, but it must be borne in mind that the majority of the nerves were near the average degree of irritability. Of the twenty-five nerves, two had become non-irritable within twenty-two hours. Curve A, figure 2, indicates the results obtained for the longer period. The abscissae represent the time in minutes or in hours, as specified, and the ordinates indicate the distance in centimeters between the primary and secondary coils.

With $\frac{M}{8}$ sodium bromide the stimulation was less than with $\frac{M}{8}$ sodium chloride. No experiments with sodium bromide were done on the large coil, but the average for those done on the small coil is shown in table 2.

TABLE 2

	TIME								
	0	½	1	2	3	4	5	7	12
Average	21.4	21.4	21.4	21.4	21.4	21.4	19.6	17.6	12.8
Maximum	21.4	21.4	21.4	21.4	21.4	21.4	21.4	21.4	21.4
Minimum	21.4	21.4	21.4	21.4	21.4	21.4	5.7	5.1	2.2

Only nine experiments were carried out with this salt. No comparisons can be made with the other sodium salts respecting the original drop in irritability, since the small coil did not register this. The bromide is evidently more toxic than the chloride, as shown in curve B, figure 3.

Sodium sulphate in $\frac{M}{12}$ concentration was more toxic than either the chloride or the bromide. The latent period of stimulation was from ten to twenty minutes, then prolonged low tetanus continued from one to three hours. The decrease in irritability was more rapid and greater than in the other sodium salts. The results of thirty-two experiments with the small coil are shown in table 3.

The greater toxicity of the sulphate is believed to be due to the sulphate ion, rather than to the higher sodium ion concentration. In the Hofmeister series sulphate is more effective than chloride, in many physical systems, so we expect it to behave as it does in physiological systems. Its toxicity is shown in curve C, figure 3.

TABLE 3

	TIME									
	0	½	1	1½	2	2½	3	5	7	12
Average	21.4	20.9	20.9	20.5	19.4	18.2	15.1	7.6	5.2	2.7
Maximum	21.4	21.4	21.4	21.4	21.4	21.4	21.4	20.0	19.2	14.5
Minimum	21.4	8.5	7.7	7.0	5.8	1.8	1.0	1.0	1.0	1.0

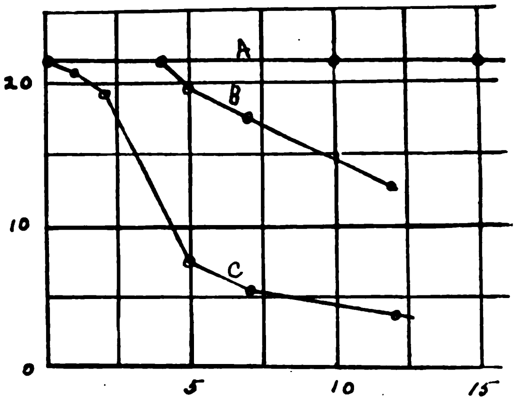

Fig. 3. A = $\frac{M}{8}$ NaCl; B = $\frac{M}{8}$ NaBr, table 2; C = $\frac{M}{12}$ Na_2SO_4, table 3

Calcium salts. The chloride was the only calcium salt used. In $\frac{M}{12}$ concentration it did not stimulate the nerve, but it did produce a medium decrease in irritability. Mathews (28) states that sodium salts are necessary for the maintenance of irritability of the nerve. The author found the nerves still irritable after being immersed in calcium chloride for sixteen hours. Calcium chloride occupies a position between sodium on the one hand, and rubidium and potassium on the other, in respect to its depressing effect. Sixty-six experiments were carried out with the small coil for three hour periods. The results are shown in table 4, also in curve B, figure 1.

TABLE 4

	TIME						
	0	50	60	90	120	150	180
Average	21.4	18.8	17.1	14.8	12.6	11.7	10.4
Maximum	21.4	21.4	21.4	21.2	21.4	21.4	18.8
Minimum	21.4	7.3	6.0	6.3	6.0	3.0	3.0

Twenty experiments were carried out with the large coil for long periods. The results are shown in table 5, and in curve B, figure 2.

TABLE 5

	TIME													
	0	½	1	2	3	4	5	7	9	10	11	12	14	18
Average	63.3	18.8	17.1	12.6	10.4	9.7	9.6	9.7	9.4	10.8	10.5	10.3	8.9	7.3
Maximum	75.0	21.4	21.4	21.4	18.8	18.1	15.6	9.0	17.1	16.4	15.9	15.0	14.0	14.0
Minimum	59.0	7.3	6.0	6.0	3.0	3.6	2.9	2.5	3.0	3.0	3.0	3.0	2.0	1.0

Thirteen out of twenty were still living at the end of fourteen hours, and three out of six were living at the end of eighteen hours.

Potassium salts. The chloride was the only potassium salt studied. An $\frac{M}{8}$ solution of potassium chloride was found to be a stimulant of the nerve, contrary to the observation made by Mathews (1). Eighty-seven experiments were carried out on this salt alone, and in about 80 per cent of the cases, stimulation occurred. The latent period was from ten seconds to one minute, and the contractions continued from two to four minutes, rarely longer. This stimulation may have been overlooked by previous investigators, but that it was due to the potassium chloride is shown by the fact that it occurred with no other salt. The decrease in irritability was rapid, and the results certainly show that rubidium and potassium chloride are similar in their toxicity effects (consult curves C and D, figs. 1 and 2).

Table 6 shows the results for three-hour periods, of eighty-seven experiments.

TABLE 6

	TIME						
	0	30	60	90	120	150	180
Average	21.4	12.3	10.7	10.2	9.4	8.4	7.4
Maximum	21.4	21.4	20.2	21.2	19.8	18.2	15.4
Minimum	21.4	5.8	2.5	3.0	3.0	2.5	1.1

Only twenty experiments were carried out on the large coil for longer periods. The results are shown in table 7.

TABLE 7

	TIME									
	0	½	1	2	3	4	5	6	7	8
Average	63.3	12.3	10.7	9.4	7.4	7.1	7.5	7.4	6.4	5.3
Maximum	75.0	21.4	20.2	19.8	15.4	13.0	12.0	9.8	8.6	7.4
Minimum	59.5	5.8	2.5	3.0	1.1	3.0	3.0	5.2	4.0	3.3

Rubidium salts. Rubidium chloride is undoubtedly a strong stimulant for nerve fiber. The latent period was found to be from ten seconds to two minutes. Twitching began, followed soon by tetanus, which continued from ten to twelve minutes. After a few single twitches the muscle relaxed and remained quiet during the remainder of the experiment. A rapid decrease in irritability was found, but a complete loss of irritability did not occur, even in twelve hours. This observation does not confirm that of Mathews, but may be attributed to a difference in technique.

Table 8 shows the results of thirty-eight experiments, on the small coil, for three hour periods.

TABLE 8
Curve D, fig. 1

	TIME						
	0	30	60	90	120	150	180
Average	21.4	9.9	7.8	7.4	7.1	6.1	5.8
Maximum	21.4	16.0	16.5	16.2	16.2	14.0	14.0
Minimum	21.4	5.5	5.2	3.0	2.5	2.0	1.0

Twenty experiments were carried out for long periods on the large coil. The results are shown in table 9, and likewise in curve D, figure 2.

TABLE 9

	TIME								
	0	½	1	2	3	4	6	8	12
Average	63.3	9.9	7.8	7.1	5.8	4.7	5.0	3.4	1.7
Maximum	75.0	16.0	16.5	16.2	14.0	6.1	8.3	7.6	8.0
Minimum	57.5	5.5	5.2	2.5	1.0	3.0	2.0	0.5	0.4

Rubidium bromide in $\frac{M}{8}$ concentration is less stimulating than rubidium chloride. It always stimulates in an $\frac{M}{4}$ solution, and occasionally in an $\frac{M}{8}$ solution. The decrease in irritability was less than with the chloride. The following table shows the results of fifteen experiments, conducted with the small coil, for long periods.

TABLE 10

Curve B, fig. 4

	TIME										
	0	½	1	1½	2	2½	3	5	8	10	12
Average..........	21.4	9.6	9.4	8.2	7.5	7.2	6.6	5.6	5.4	5.6	2.5
Maximum........	21.4	13.3	13.0	11.0	10.2	10.4	9.4	8.5	9.5	9.2	5.6
Minimum.........	21.4	6.8	6.5	5.2	3.8	5.2	4.4	2.6	1.7	2.5	0.8

Rubidium sulphate is a stronger stimulant than the chloride, and the toxicity is greater. The action of the rubidium sulphate bears a relation to the actions of rubidium chloride and rubidium bromide similar to the relation between sodium sulphate and the chloride and bromide. Table 11 gives the average for eight experiments.

TABLE 11

Curve C, fig. 4

	TIME									
	0	½	1	1½	2	2½	3	6	7	8
Average................	21.4	10.2	8.5	6.3	6.1	4.9	4.3	4.4	3.2	2.4
Maximum..............	21.4	18.7	17.1	13.8	10.3	9.4	7.3	5.0	4.2	3.6
Minimum..............	21.4	6.6	6.1	4.8	2.9	1.2	1.2	3.4	2.0	1.0

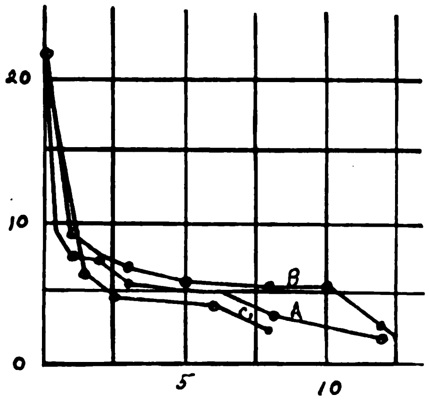

Fig. 4. A = $\frac{M}{8}$ RbCl, table 9; B = $\frac{M}{8}$ RbBr, table 10: C = $\frac{M}{12}$ Rb_2SO_4, table 11

2. *Combinations of three salts*

Investigations were carried out in which rubidium was used instead of potassium in Ringer's solution. The Ringer's solution used as a control contained 7 grams of sodium chloride plus 1 cc. of 2.5 M KCl plus 1 cc. of 2.5 M $CaCl_2$ per liter. The modified Ringer's solution contained the same amounts of sodium and calcium chlorides, and 1 cc. 2.5 M RbCl per liter, instead of the same amount of KCl. The following table shows the results of twenty experiments, conducted with the large coil, for long periods:

TABLE 12

Curve B, fig. 5

	TIME								
	0	½	1	1½	2	2½	3	7	18
Average	63.3	55.8	54.8	53.5	52.7	52.7	53.1	55.0	35.9
Maximum	75.0	78.5	71.5	62.5	63.0	63.0	63.0	65.0	48.5
Minimum	45.0	45.0	45.0	45.0	44.0	44.0	44.0	13.5	13.5

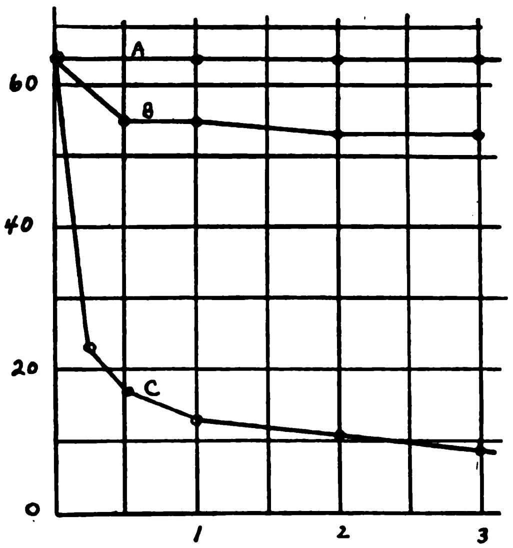

Fig. 5. A = Controls in Ringer; B = Ringer—K substituted by Rb, table 12; C = Ringer—Na substituted by Rb, table 13.

This shows that rubidium is certainly closely related to potassium, since the nerves lose very little in irritability within several hours, in a solution containing rubidium in place of potassium.

Another series of experiments was carried out in which rubidium was used instead of sodium in Ringer's solution. This solution contained 119.6 cc. of $\frac{M}{1}$ RbCl per liter, 1 cc. of 2.5 M $CaCl_2$, and 1 cc. of 2.5 M KCl. This modified Ringer was found very toxic, as shown in table 13, and again in curve C, figure 5, where the results of twenty-four experiments are given. Certainly rubidium is not similar in its action to sodium, else it could replace it in a balanced solution.

TABLE 13

	TIME							
	0	¼	½	1	1½	2	2½	3
Average	63.3	23.7	16.7	12.3	11.7	10.2	9.7	8.4
Maximum	75.0	50.0	30.0	23.5	22.0	19.0	18.0	16.0
Minimum	57.5	10.5	6.0	6.0	5.5	5.0	5.5	5.5

Curve A, figure 5, represents the controls in Ringer's solution. As these were run with daily observations a large number was obtained, but the curve shown may be considered to represent twenty. With frogs in good condition there was seldom any loss in irritability for twenty hours, as before stated. From these experiments alone, rubidium is shown to be most similar to potassium in its physiological behavior.

3. Hypotonic solutions

A series of experiments was performed with varying concentrations of rubidium salts. In one series a concentration of twentieth or fortieth molecular was used, knowing that the solution was hypotonic. In the other series a similar quantity of rubidium salt was used, but a final osmotic pressure equivalent to an eight-molar solution was obtained by the addition of the proper amount of saccharose. The results for $\frac{M}{20}$ RbCl in saccharose are shown in table 14 and in curve A, figure 6. Twenty experiments were performed.

TABLE 14

	TIME						
	0	30	60	90	120	150	180
Average	21.4	19.9	18.7	16.3	14.3	12.4	11.1
Maximum	21.4	21.4	21.4	21.4	21.4	21.4	21.4
Minimum	21.4	11.0	12.0	9.4	8.4	8.0	6.8

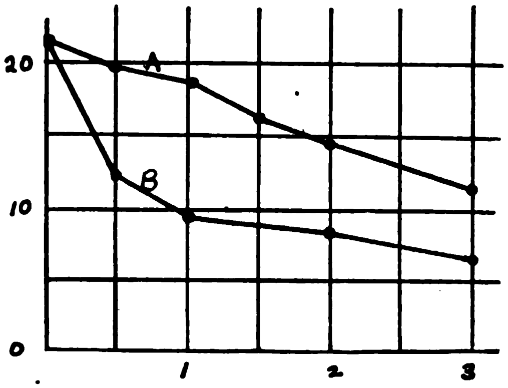

Fig. 6. A = $\frac{M}{20}$ RbCl in sacc., table 14; B = $\frac{M}{20}$ RbCl in H_2O, table 15

Eight experiments were carried out with $\frac{M}{20}$ RbCl in water, and table 15, also curve B, figure 6, show the effects of the hypotonic solution. Osmotic pressure appears to have some effect.

TABLE 15

	TIME						
	0	30	60	90	120	150	180
Average	21.4	12.1	9.3	8.4	8.1	7.8	6.5
Maximum	21.4	21.4	14.3	16.7	12.6	12.7	12.1
Minimum	21.4	6.0	6.0	3.6	3.8	3.6	1.2

The results of thirteen experiments with $\frac{M}{20}$ Rb_2SO_4 in saccharose are shown in table 16, and again in curve A, figure 7.

TABLE 16

	TIME						
	0	30	60	90	120	150	180
Average	21.4	16.9	14.7	10.7	10.3	9.1	8.8
Maximum	21.4	21.4	21.4	21.4	20.3	19.0	18.0
Minimum	21.4	9.5	8.8	8.2	6.1	4.8	1.1

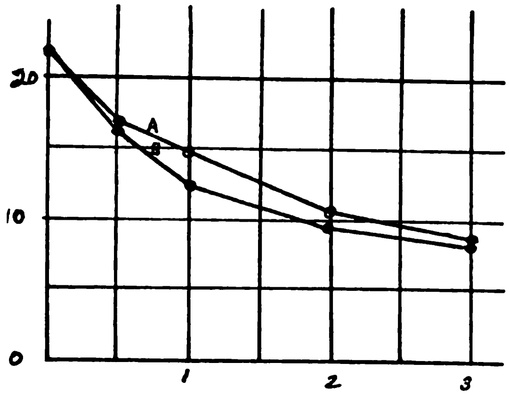

Fig. 7. A = $\frac{M}{20}$ Rb_2SO_4 in sacc., table 16; B = $\frac{M}{20}$ Rb_2SO_4 in H_2O, table 17

The results of six experiments with $\frac{M}{20}$ Rb_2SO_4 in water are shown in table 17, and in curve B, figure 7.

TABLE 17

	TIME						
	0	30	60	90	120	150	180
Average	21.4	16.2	12.3	10.6	9.3	8.7	8.2
Maximum	21.4	21.4	18.7	15.5	15.0	14.7	14.2
Minimum	21.4	11.1	9.9	8.7	5.9	6.5	5.8

This again shows a slightly higher irritability with the isotonic solution.

A very few experiments were done with $\frac{M}{40}$ Rb_2SO_4 in saccharose, the results of which are shown in table 18 and in curve A, figure 8. The corresponding nerves in $\frac{M}{40}$ Rb_2SO_4 in water gave lower results, as shown in table 19 and in curve B, figure 8.

TABLE 18

	TIME						
	0	30	60	90	120	150	180
Average	21.4	18.2	17.6	15.5	11.9	11.5	10.7
Maximum	21.4	21.4	21.4	21.4	18.2	18.8	18.1
Minimum	21.4	11.8	11.4	6.5	6.5	6.8	6.2

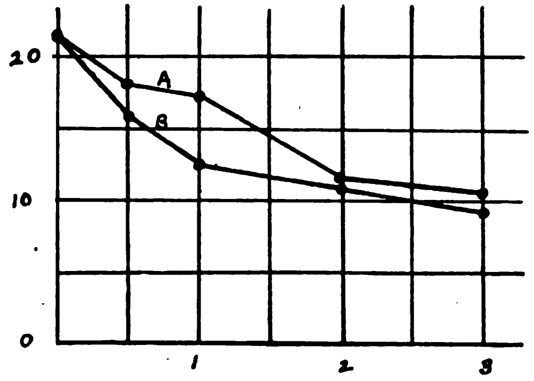

Fig. 8. A = $\frac{M}{40}$ Rb_2SO_4 in sacc., table 18; B = $\frac{M}{40}$ Rb_2SO_4 in H_2O, table 19

TABLE 19

	TIME						
	0	30	60	90	120	150	180
Average	21.4	16.0	12.6	11.7	11.3	10.3	9.1
Maximum	21.4	21.4	15.7	13.7	13.9	12.7	11.3
Minimum	21.4	10.6	8.7	8.7	9.7	8.6	7.5

This shows that the isotonic solution preserves a slightly higher irritability than the hypotonic solution. These experiments are by no means exhaustive, yet they indicate that isotonic solutions are less toxic than hypotonic, or that osmotic pressure is a possible factor in maintaining the irritability of nerves in various solutions.

4. Salt antagonisms

A series of investigations was made with combinations of two salts, for the purpose of testing the so-called antagonistic action. For these investigations 1 part of $\frac{M}{8}$ calcium chloride and 99 parts of $\frac{M}{8}$ sodium, potassium or rubidium chloride were used throughout. Twenty-one long experiments were carried out with the mixture of calcium and sodium. Undoubtedly the calcium chloride in this concentration had some antagonizing action, as shown in tables 1 and 20 and in curves A and B, figure 9.

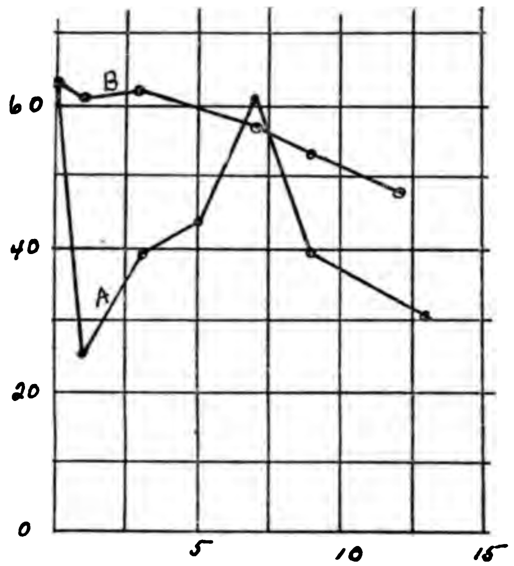

Fig. 9. A = $\frac{M}{8}$ NaCl, table 1; B = 99 parts $\frac{M}{8}$ NaCl, plus 1 part $\frac{M}{8}$ $CaCl_2$, table 20.

TABLE 20

	TIME										
	0	½	1	2	3	5	7	8	9	10	12
Average..........	63.3	60.9	60.8	61.4	62.0	59.8	57.1	56.9	53.0	49.9	48.0
Maximum........	75.0	68.5	68.5	68.5	69.0	69.0	65.4	66.0	67.0	66.5	69.5
Minimum.........	52.5	52.5	53.0	53.0	55.0	51.0	44.0	35.0	25.5	9.0	4.2

The initial fall in irritability in $\frac{M}{8}$ sodium chloride is prevented by the addition of a small amount of calcium, and even after twelve hours the nerve in the mixture is much more irritable than the one in sodium chloride alone. This surely indicates a protective action of some kind on the part of calcium.

The picture obtained by using 1 part of $\frac{M}{8}$ calcium chloride and 99 parts of $\frac{M}{8}$ potassium chloride is quite different from the above picture. Twenty-three experiments were carried out with this combination. The calcium here played a minor rôle, and its protective action was not a decided one. The results are shown in tables 6 and 21, and in curves A and B of figure 10. The decreases in irritability of the two series were similar, the addition of calcium being slightly beneficial.

TABLE 21

	TIME				
	0	½	1	2	3
Average	63.3	14.9	13.3	10.5	9.0
Maximum	75.0	28.0	23.0	19.5	17.5
Minimum	55.0	7.5	6.7	4.0	2.5

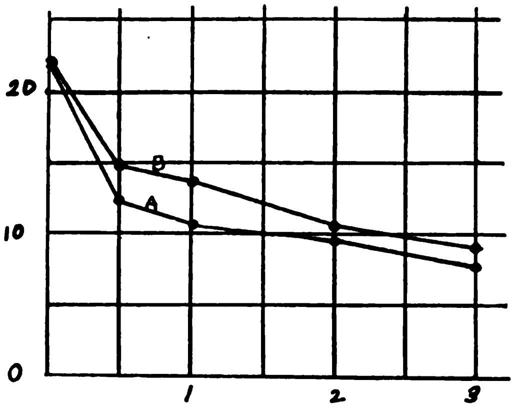

Fig. 10. A = $\frac{M}{8}$ KCl, table 6; B = 99 parts $\frac{M}{8}$ KCl plus 1 part $\frac{M}{8}$ $CaCl_2$, table 21.

Twenty-five experiments were carried out with a similar mixture of calcium and rubidium chlorides. The results were much like those obtained with the mixture of calcium and potassium chlorides. The calcium showed a slight protective action. The results are shown in tables 8 and 22 and in curves A and B of figure 11.

TABLE 22

	TIME				
	0	½	1	2	3
Average	63.3	13.1	10.8	9.3	7.9
Maximum	75.0	21.0	20.0	18.5	17.0
Minimum	55.0	5.0	4.0	2.5	2.0

It will be noticed that rubidium and potassium behave similarly in respect to the effects obtained when these are combined with a small amount of calcium. Neither is antagonized to a great extent, while in the case of sodium a decided antagonism exists.

The question of the part played by the radio-activity of rubidium has been considered by some authors. Henriot (26) states that rubidium emits a radiation giving a current of 3.64×10^{-13} amp. per sq. cm. of surface of rubidium sulphate, while in the same circumstance the radiation of potassium is only 2.74×10^{-13} amp. The rays are more intense and more penetrating than those of potassium. The radio-activities of the salts of potassium and rubidium probably serve to differentiate them from the salts of sodium and calcium, in physiological effects, but facilities are not at hand for further investigation in this direction.

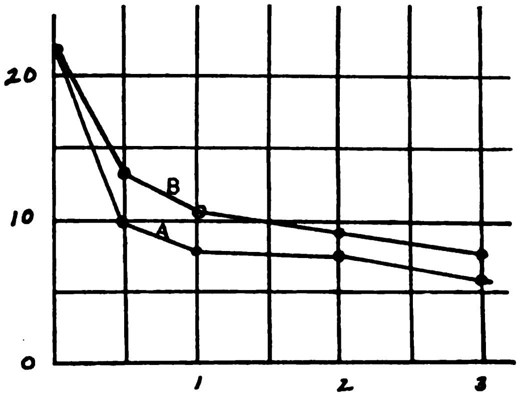

Fig. 11. A = $\frac{M}{8}$ RbCl, table 8; B = 99 parts $\frac{M}{8}$ RbCl plus 1 part $\frac{M}{8}$ $CaCl_2$, table 22.

5. *Additional observations*

It appeared highly desirable to substantiate the physiological effects obtained above by results on non-living systems. A minor study of the comparative effects of sodium, potassium, and rubidium salts on the precipitation of lecithin and kephalin emulsions was carried out. Since the myelin sheaths of nerve fibers are composed largely of lecithin and kephalin and since the sodium and potassium contents vary considerably in lecithin and kephalin (27), it was supposed that